RMEP 1994-031

Journal of Chemical Ecology, Vol. 20, No. 7, 1994

WHY ARE PREDATOR URINES AVERSIVE TO PREY?

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(Received October 12, 1993; accepted January 31, 1994)

Abstract—Predator odors often repel prey species. In the present experiments, we investigated whether changes in the diet of a predator, the coyote (*Canis latrans*) would affect the repellency of its urine. Furthermore, because predator odors have a high sulfur content, reflecting large amounts of meat in the diet, we investigated the contribution of sulfurous odors to repellency. Our results were consistent with the hypothesis that diet composition and sulfurous metabolites of meat digestion are important for the repellency of predator odors to potential prey.

Key Words—*Aplodontia rufa*, avoidance, *Canis latrans*, *Cavia porcellus*, coyote, guinea pig, mouse, mountain beaver, *Mus musculus*, predator odors, *Peromyscus maniculatus*, urine.

INTRODUCTION

Predator odors are generally aversive to potential prey species, including *Lepus* and *Cuniculus* (Sullivan et al., 1985a; Sullivan, 1986; Sullivan and Crump, 1984, 1986a; Robinson, 1990), *Aplodontia* (Epple et al., 1993; Nolte et al., 1993), *Microtus* (Dickman and Doncaster, 1984; Gorman, 1984; Stoddart, 1976,

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0098-0331/94/0700-1505$07.00/0

1980, 1982; Sullivan et al., 1988; Merkins et al., 1991), *Cethrionomys* and *Apodemus* (Robinson, 1990; Stoddart, 1980), *Thomomys* (Sullivan et al., 1990; Sullivan and Crump, 1986b), *Marmota* (Swihart, 1991), *Rattus* (Vernet-Maury, 1980; Vernet-Maury et al., 1984), *Capreolus* and *Cervus* (Abbott et al., 1990; Van Haaften, 1963), and *Odocoileus* (Melchiors and Leslie, 1985; Muller-Schwarze, 1972; Sullivan et al., 1985b; Swihart et al., 1991). Avoidance appears to be mediated, at least in part, by urinary constituents that are not species specific. Such compounds may constitute a generalized meat-eater cue (Epple et al., 1993; Abbott et al., 1990) or a predator "leitmotif" (Stoddart, 1980). Although the chemical nature of this leitmotif remains obscure, one possibility is that it features odoriferous constituents that reflect the diet composition of the predator. Such odors might include sulfur-containing metabolites of protein digestion (Mason et al., 1993). We conducted the present experiments to assess whether diet manipulations would affect the repellency of urine from a predator to several potential prey species and to investigate the contribution of sulfurous compounds to its repellency.

METHODS AND MATERIALS

Experiment 1

This experiment was conducted to determine whether diet manipulations would affect the repellency of a predator urine to several prey species.

Subjects. Four rodent species: mountain beaver (*Aplodontia rufa*, $N = 9$), house mice (*Mus musculus*, $N = 40$), deer mice (*Peromyscus maniculatus*, $N = 20$), and guinea pigs (*Cavia porcellus*, $N = 12$) served as subjects. The two species of mice and the guinea pigs were bred and tested at the Monell Chemical Senses Center. Each animal was individually caged (mice, $27 \times 21 \times 14$ cm; guinea pigs, $50 \times 50 \times 30$ cm) under a 12L:12D cycle (light onset at 0700 hr). The mountain beavers were trapped in the vicinity of Olympia, Washington. These animals were housed and tested in outdoor pens ($3 \times 3 \times 1.5$ m) at the Denver Wildlife Research Center (DWRC) Olympia, Washington, research facility.

Stimuli. Four male coyotes (*Canis latrans*) at the DWRC research facility in Millville, Utah, were randomly selected from the captive colony to serve as urine donors. During the first two weeks of July 1992, these animals were maintained exclusively on a diet of cantaloupe. On days 15–20 they were placed in metabolism chambers ($120 \times 70 \times 80$ cm) for 18 hr and urine samples were collected (FU). These samples were pooled and frozen at 40°C. On day 21, these four coyotes were returned to their normal diet of minced raw meat for a two-week period. After this two-week period, additional urine samples (MU) were collected on each of five days, as described above. These MU samples were also pooled and frozen. Subsequently, FU and MU samples were shipped to the Monell Chemical Senses Center and the DWRC Olympia, Washington, facility for chemical and behavioral tests.

Procedures. Similar testing methods were used with all species. Urine samples (1 ml) were pipetted onto pieces of absorbent paper placed inside small (38-mm-diam. × 8-mm) perforated plastic containers. For mice, the containers were placed inside aluminum weigh-boats and the assemblies were secured 10 cm apart with thumbtacks to pieces of wood. Forty sunflower seeds were then weighed and placed in each weigh-boat around the plastic containers. For guinea pigs, the perforated plastic containers were put inside the animals' food cups, and surrounded with 20 g of guinea pig chow. The cups were placed 10 cm apart in the guinea pigs' cages. During mountain beaver tests, the perforated containers were placed inside weigh-boats and surrounded with 10 1-cm^3 apple cubes. The assemblies were then secured 10 cm apart to the ground with metal stakes.

Mice and guinea pigs were adapted to an 18-hr food deprivation schedule. On each of two pretreatment days, these animals were given their respective foods for 2 hr in weigh-boats/food cups containing perforated containers with a piece of absorbent paper treated with 1 ml of tap water. On the two treatment days that followed, the animals were given the same foods; however, this time the absorbent paper was treated with 1 ml of either FU or MU. At the end of each test session, the amount of food remaining in each weigh-boat/food cup was assessed.

Unlike the other three species, mountain beavers were not food deprived because they do not readily adapt to deprivation regimes. On two pretreatment days, all animals were presented with apple cubes placed in the weigh-boats with tap-water-treated absorbent paper in perforated containers. On the two treatment days that followed, the animals were given 24-hr, two-choice tests between apple cubes associated with either FU or MU. The number of cubes remaining after 3, 6, 12, and 24 hr was assessed. Since some animals did not respond until late in the 24-hr period, and because the pattern of results was similar at each measurement interval, only the 24-hr results were presented. Apple weights were not evaluated because moisture loss or gain could not be accurately assessed.

For all individuals of each species, the left–right position of FU urine samples was randomly determined on day 1, and then reversed on day 2.

Analyses. The data for each species was evaluated separately in a two-factor repeated measures analysis of variance (ANOVA). In each case, urine type was the main effect, with the animals nested within urine type and the repeated measure was days. Tukey tests (Winer, 1971, p. 201) were used to isolate significant differences among means subsequent to the omnibus procedure ($P < 0.05$).

Results. All species ingested more (mountain beaver, $P = 0.0064$; house mice, $P = 0.0002$; deer mice $P = 0.0082$; guinea pigs $P = 0.0444$) food from bowls containing FU than they did from bowls scented with MU (Figure 1). There were no day effects ($P > 0.25$) and no interactions between urine types and days ($P > 0.25$).

Experiment 2

Differences in animal response between FU and MU samples in the first experiment may have reflected dilution effects. To control for this possibility, FU and MU samples were lyophilized and then rehydrated to a common concentration. Because all species showed similar responses in experiment 1, deer mice were arbitrarily selected to serve as subjects.

Subjects. Experimentally naive deer mice ($N = 14$) served as subjects.

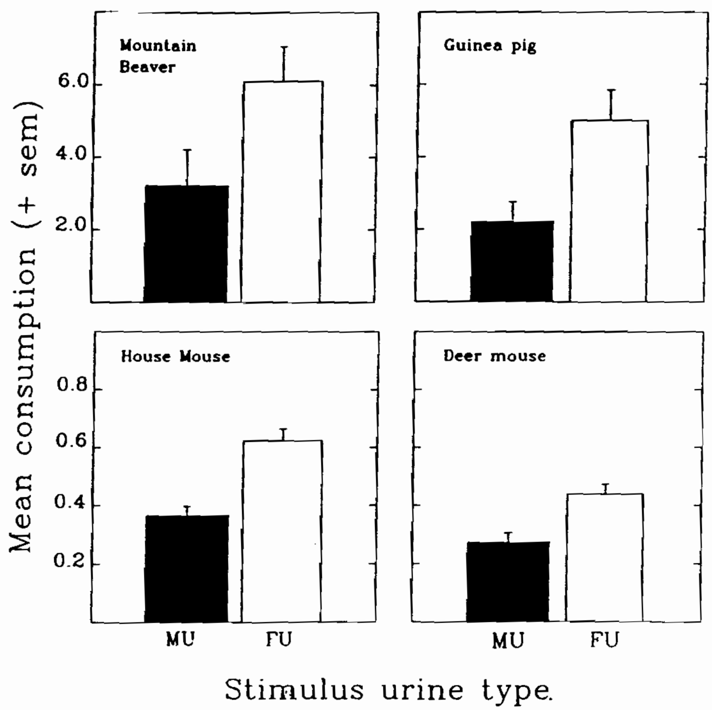


FIG. 1. Intake by mountain beavers, guinea pigs, house mice, and deer mice of food (number of apples cubes, grams of guinea pig chow, grams of sunflower seeds, grams of sunflower seeds; respectively) associated with urine collected either from coyotes on a fruit diet (FU) or from coyotes fed meat (MU).

Animals were individually caged (27 × 21 × 14 cm) under a 12L:12D cycle (light onset at 0700 hr).

Stimuli. FU and MU were the same as used in experiment 1 except, prior to testing, the samples were lyophilized and then rehydrated to a common concentration.

Procedures. Test apparatus was the same as described for experiment 1. On each of four pretreatment days, food-deprived mice (18 hr) were presented with two weigh-boats, each containing 40 sunflower seeds of known weight, and a perforated plastic container with an enclosed absorbent paper treated with 1 ml of tap water. After 2 hr, the weight-boats were removed and the number of seeds remaining were assessed. On the basis of ingestion, animals were then assigned to two counterbalanced groups. Four treatment days immediately followed. One group ($N = 8$) was given 2-hr tests offering choices between sunflower seeds associated with FU or tap water. The other group ($N = 6$) was given 2-hr tests offering choices between sunflower seeds associated with MU or tap water.

Analysis. Difference scores were calculated by subtracting FU or MU ingestion from control (tap-water-scented) ingestion by individual deer mice. Therefore, high positive values indicate avoidance of the urine stimuli relative to the control, while negative values indicate preference for the urine stimuli. These data were evaluated in a two-factor repeated measures ANOVA. Tukey post-hoc tests were used to isolate differences among means ($P < 0.05$).

Results. Mice ingested relatively more ($P = 0.0030$) from FU-scented bowls than from MU-scented bowls (Figure 2). There were no day effects ($P > 0.05$) or interaction between urine types and days ($P > 0.05$).

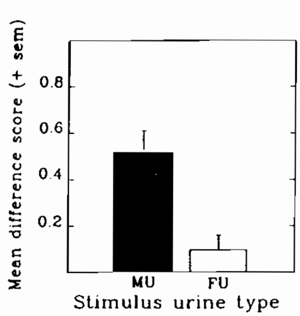


FIG. 2. Difference scores calculated by subtracting ingestion by deer mice of sunflower seeds associated with urine from coyotes on a fruit diet (FU) or urine from coyotes on a meat diet (MU) from ingestion of sunflower seeds associated with a control stimuli (tap water) during two-choice tests.

Experiment 3

Diet manipulation experiments showed that the presence/absence of meat in a donor coyote's diet affected the repellency of the urine. This result led us to hypothesize that by-products of meat digestion in urine, such as sulfur compounds, might contribute to aversiveness. To test this hypothesis, we removed sulfur-containing substances from MU by precipitation with mercuric chloride. The responses of mountain beaver to urine stimuli with and without sulfur compounds were then investigated.

Subjects. Experimentally naive mountain beaver ($N = 10$) were obtained and maintained as described in experiment 1.

Stimuli. MU was the same as described for experiment 1. Sulfur-free urine (SR) was prepared by precipitating MU with mercuric chloride (Golovnya et al., 1972). Briefly, 319 mg of 1 mmol mercuric chloride was dissolved in 2 ml of methanol and added to 25-ml samples of MU. The mixture was agitated for 30 min, stored for 3 hr at room temperature (23°C), and finally, centrifuged. The centrifugate was collected for behavioral testing.

Procedure. Test apparatus and procedures were similar to those described for mountain beaver in experiment 1. Counterbalanced two-choice tests were used to assess ingestion of apple cubes associated with either tap water or MU, and apple cubes associated with either tap water or SR. For each test, apple cubes were available for 12 hr on two consecutive days.

Analysis. Difference scores were calculated by subtracting the number of apple cubes associated with MU or SR ingested by mountain beaver from the number of apple cubes associated with tap water ingested during the same trial. Therefore, high scores indicate a relative avoidance of the urine stimuli, while low scores indicate an indifference. Subsequently, these data were evaluated in a two-factor repeated measures ANOVA. Tukey post-hoc tests were used to isolate differences among means ($P < 0.05$).

Results. Sulfur-free urine samples from meat-fed coyotes were less offensive ($P = 0.0016$) to mountain beavers than whole urine from coyotes fed meat (Figure 3). There were no day effects ($P > 0.05$) or an interaction between urine types and days ($P > 0.05$).

Urine Fractionation and Chromatography

The first two experiments indicated that the donor coyote's diet affected the aversiveness of urine. In the third experiment, urine from meat-fed coyotes precipitated with mercuric chloride was less offensive to mountain beaver than was the whole urine. Chromatograms were subsequently prepared to depict changes in sulfur constituents of these test stimuli.

Stimuli. FU and MU samples were the same as used in experiment 1, and mercury precipitation was as described for experiment 3.

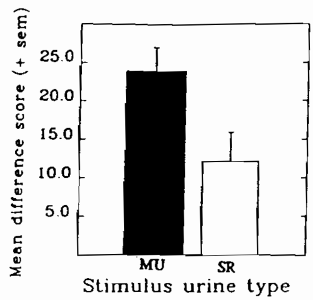


FIG. 3. Difference scores calculated by subtracting ingestion by mountain beavers of apple cubes associated with urine from coyotes on a meat diet (MU) or sulfur-free urine from coyotes on a meat diet (SR) from ingestion of apple cubes associated with a control stimuli (tap water) during two-choice tests.

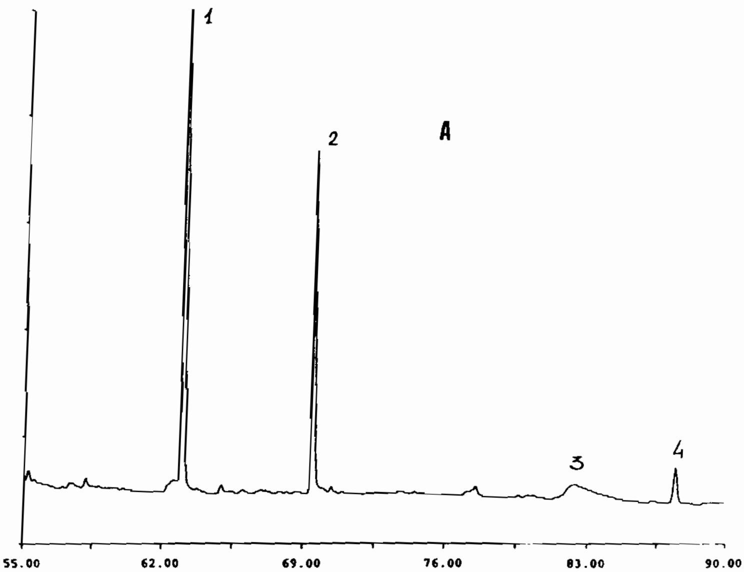
Methanol Fractionation. Coyote urine (12 ml) was applied to a glass chromatographic column (1 cm ID, 40 cm) packed with Kiselgel RP-18 (40–63 μm; Merck) previously washed with 40 ml of methanol and 40 ml of distilled water. Hydrophilic components of the urine were eluted with distilled water until the current eluate showed a neutral pH; the column was then dried by passing compressed nitrogen through it, and hydrophobic compounds were eluted with methanol (20 ml). The resultant methanol fractions were then removed.

Analytical HPLC. Analytical HPLC of all methanol fractions was performed using a Rainin HPXL two-pump solvent delivery system, Zorbax OS (4.6 × 250 mm) column, and Dynamax UV-M detector. The analytical conditions were: mobile phase: 0–5 min isocratic acetonitrile water 5:95, 5–40 min linear gradient to acetonitrile water 4:6, 40–60 min linear gradient to 100% acetonitrile, 60–80 min isocratic 100% acetonitrile; detection at 204 nm; injection volume, 100 μl.

Results. Among the four major constituents on chromatograms of MU and FU, there were only three peaks common to both urine types (Figure 4). Two of these peaks disappeared from MU after treatment with mercuric chloride (Figure 5).

DISCUSSION

The aversiveness of coyote urine to herbivorous rodents fluctuated with a change in the predator's diet. All species tested ingested more food from bowls scented with urine collected for coyotes fed cantaloupe than they did from bowls scented with urine from coyotes on a meat ration. Deer mice also showed similar tendencies in the second experiment, an indication that reduced repellency reflected a urine solute constituent change rather than merely a dilution effect.



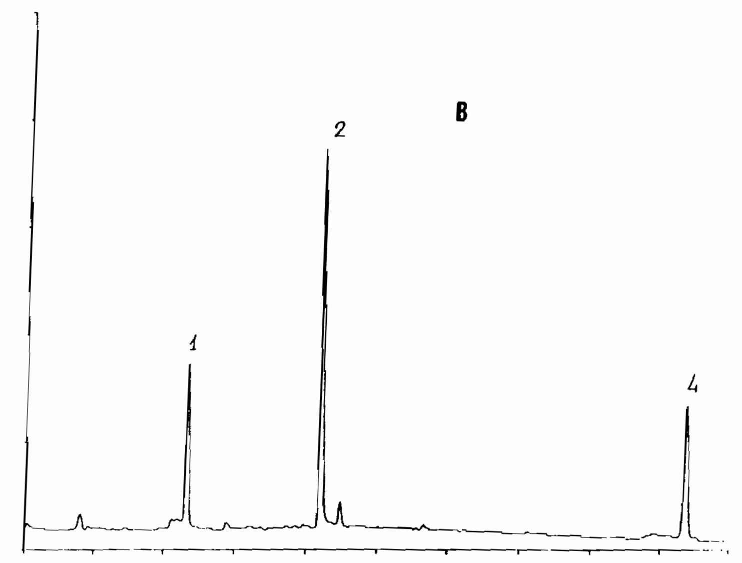


FIG. 4. Chromatograms of urine (methanol fractions) collected from coyotes (A) on a fruit diet (FU) or (B) on a meat diet (MU).

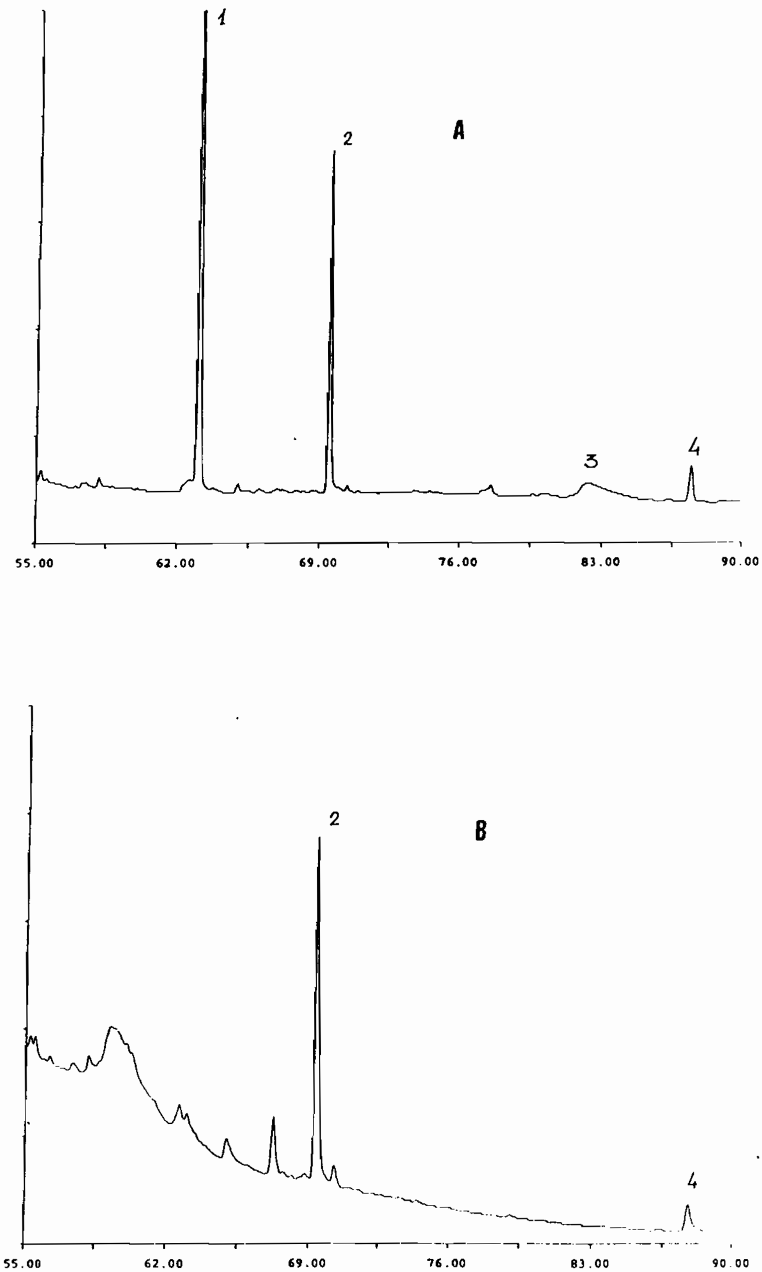


FIG. 5. Chromatograms of urine (methanol fractions) from meat-fed coyotes before (A) and after (B) treatment with mercuric chloride.

The appearance and/or maintenance of sulfur compounds in coyote urine appears to be correlated with repellency. MU treated with mercuric chloride to remove sulfur compounds was less aversive to mountain beavers than untreated samples. Further, sulfur constituents are a primary difference between MU and FU samples. The major component of MU (peak 1, Figure 4) is a minor component in FU, and at least one other constituent of MU (peak 3) is absent from FU. Both of these peaks disappeared from MU after it was treated with mercuric chloride (Figure 5), indicating that they were sulfur-containing.

Nearly half the volatile material in coyote urine is methyl (2-methyl-2-butenyl) sulfide (MMBS) (Schultz et al., 1988), and this substance is common in other predator urines as well (Raymer et al., 1988; Jorgenson et al., 1978). However, the retention time of synthetic MMBS did not correspond to the retention times of peaks 1 or 3 for MU. Although these peaks are probably not MMBS, they are as yet unknown.

FU and SR were both less aversive than MU; however, food intake was reduced in the presence of FU and in the presence of SR relative to control stimuli. Prior experiments indicate that unfamiliar but behaviorally irrelevant odors (e.g., butyric acid, guinea pig urine) do not inhibit feeding by mountain beaver (Epple et al., 1993). Therefore, this weak avoidance may be attributed to other nonsulfurous compounds. For example, acetophenones occur in mustelid (Sullivan and Crump, 1986b) and wolf (Raymer et al., 1986) scents. ortho Aminoacetophenone (OAP) reduces forage intake by mountain beaver (Nolte et al., 1993). Avoidance of plants treated with OAP, however, is considerably less than mountain beaver avoidance of the same plants treated with whole coyote urine (Nolte et al., 1993).

Overall, the present results are consistent with the hypothesis that (as yet unknown) sulfurous odors associated with meat digestion are important for the repellency of predator urines to potential prey. A plausible speculation is that prey use such odors as cues to the diet composition of donors. It may also be that predators attend to sulfurous urinary odors and interpret them as indicators of diet composition; this provides an explanation of the attractiveness of predator urines to these species (Bullard et al., 1983; Fagre et al., 1982).

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